POST HARVEST ROT OF FRUITS AND VEGETABLES

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A C K N O W L E D G E M E N T

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(Pammie Joshi)

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INTRODUCTION AND REVIEW OF LITERATURE

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Fruits and vegetables form an important component of the diet of vegetarians providing sources of vitamins and minerals. This is all the more true for India where a majority of the population is vegetarian.

Although exact data on the annual production of fruits and vegetables in India are not available but it is estimated that acreage under cultivation ranges between 28-30 million acres. The produce is not sufficient enough for consumption of the people partly because major part is destroyed by pests and diseases including post harvest during handling, transportation and storage.

Powell (1906) named this discipline as Market pathology, dealing with diseases affecting fresh fruits and vegetables after the harvest and before their consumption. Broadly speaking the science is concerned with the spoilage occuring during the operations of harvesting, packing, handling, transportation, storing the produce.

According to Bourne (1970) the loss of food during post harvest can be defined as "that weight of wholesome edible product (exclusive of moisture content) that is normally consumed by human and that has been seperated from the medium and the site of its immediate growth or production by deliberate human action with the intention of using it for human feeding but which for any reasom fails to be consumed by humans".

Over all losses to fruits and vegetables from the farms to the consumers are about 12% (Columbie, 1964, Lentz & Vaselen Berg 1971) of the total production. According to U.S.D.A. estimates (1965) losses of the kind in fruits nuts, vegetables are to the tune of 23%.

In India Chenulu & Thakur (1968) while conducting a survey of the extent of losses estimated that potatoes suffer a loss of 24.0%, tomato 19.3%, mango 17.7%, banana 13.5% and apple 10.8%. Harvey (1970) while reviewing the literature of post harvest losses pointed out that the total percent loss in apples were 1.7-3.6, cucumber 2.9-7.9, grapes 5.0-10.5, lettuce 4.1-11.7, oranges 3.2-4.2, peaches 2.3-12.6, pears 4.1 - 10.1 and tomato 11.4 - 14.2. By and large the losses are much more in developing countries like India than in developed countries because of lack of advanced technology. Onesirosan and Fatunla (1976) showed that upto 21% of the potential harvest of tomato fruits were lost to rots in the field and an additional 5 - 20% rotted during marketing. Parpia (1976) on the other hand observed that losses during storage and handling of all crops in tropical Africa were to the extent of more than 30%. Thus it is now realised that post harvest diseases cause considerable losses to fruits and vegetables round the globe.

Large number of post harvest disease of various fruits and vegetables have been reported from time to time and some of the important ones are listed in Table I.

TABLE - I

List of some of the important post harvest diseases of fruits and vegetables.

Name of the fruit/ vegetable	Name of the fungus	References
1	2	3
Aegle marmelos (Bel)	Fusarium solani (Mart) App & Wr	Sharma et al (1979)
	Phoma glomerata (Corda) Wr & Hochapfel	Pandey et al (1980)
Annona squamosa L.	Glomerella cingulata	Dhingra et al (1981 b)
Averrhoa carambola L. (Kamrakh)	Cladosporium herbarum (Pers) Link	Sharma & Khan (1978)
Carica papaya L.	Pythium aphanidermatum	Bhargava (1941)
	Rhizopus stolonifer	Tandon & Mishra (1969)
	Fusarium solani (Mart) App & Wr	Quimo & Quimo (1978)
	Corynespora cassiicola	Quimo & Abilay (1981)
	Phytophthora capsici	Aragaki & Uchida (1979)
	Colletotrichum capsici (Syd) Butler & Bisby	Lal et al (1980)
	Aspergillus niger van Tiegh	Garcha & Singh (1980)
	Trichothecum roseum Link	Saxena & Jain (1981)
Carissa Carandas	Myrotheciam roridum Tode ex Fr	Kumar & Tandon (1979)
	Cochliobolus spicifer Nelson	Wadia & Manoharachary (1979)
	Rhizoctonia solani Kuehn	
Citrullus lanatus	Fusarium solani (Mart) App & Wr	Ikediugwu & Ogieva (1979)
	Corticium rolfsii	Ikediugwu (1981)

1	2	3
Citrullus vulgaris	*Fusarium oxysporum* (Schl ex Fr) em Snyder	Mathur & Mathur (1958)
		Suryanarayana & Nath (1963)
	Fusarium equiseti (Corda) Sacc	Chaurasia (1980)
Citrus sp	*Aspergillus niger* van Tiegh	Srivastava & Tandon (1969)
	Trichothecium roseum Link	Cheema & Jeyarajan (1972)
	Geotrichum candidum Link ex Pers	Kamal *et al* (1978)
	Phytophthora citrophthora *P.* (nicotianae var) *parasitica* (Dastur) *P. syringae* *P. hibernalis*	Feld *et al* (1979)
	Trichoderma lignorum (Tode) Harz	Kanaujia (1979)
	Penicillium dicitatum Sacc *P. italicum* Wehmeyer *Botrytis cinerea* (Pers) Link *Sclerotinia sclerotiorum* (Lib) de Bary	Dai (1979)
	Penicillium dicitatum Sacc *P. italicum* Wehmeyer *Diplodia natalensis* (Botryodiapodia theobromae) *Colletotrichum gloeosporoides*	Kumbhare and Choudhari (1980)

1	2	3
	Alternaria alternata (Fr) Keissler	Garcha & Singh (1980)
	Aspergillus fumigatus Thom	
	As. niger van Tiegh	
	Botryodiplodia theobromae Pat	
	Fusarium semitectum Berk & Rav	
	Penicillium crustosum	
	Pa. cyclosporium	
	Rhizopus stolonifer	
	Aspergillus aculeatus Iis	Kanaujia (1981 a)
	Trichoderma viride Pers ex Fr	Kanaujia (1981 b)
	Hormonema dematioides	Rajak & Gautam (1981)
	Dreschslera (Sctosphaeria) rustrata	
	Acremonium cymosum	Dhingra et al (1981a)
Fragaria virginiana (Strawberry)	Colletotrichum fragariae Brooks	Howard (1972)
	Dendrophoma obscurans (Ell & Ev)	Howard & Albregts (1973)
	Pestalotia longisetula	Howard & Albregts (1974a)
	Alternaria tenuissima (Nees ex Fr)	Howard & Albregts (1974b)
	Phytophthora nicotianae Var parasitica (Dastur)	Matsuzaki et al (1981)
		Suzvi et al (1981)
	Phytophthora cactorum (Leb & Cohn)	Kao & Lev (1981)
	Ps. citrophthora	

1	2	3
	Mucor sp Rhizopus sp Botrytis cinerea	Dennis (1981)
Litchi chinensis (Litchi)	Geotrichum candidum Link ex. Fr	Jamaluddin et al (1978)
Malus (Apple)	Trichothecium roseum (Pers) Link ex Fr	Agrawala & Sharma (1968)
		Sree Kantilal et al (1974)
	Hormiscium sp	Jones & Fields (1972)
	Pestalotia sp	Mathur & Agarwal (1974)
	Glomerella cingulata	Brook (1978)
	Aspergillus candidus Link	Thind et al (1978)
	Phytophthora syringae	Bolay (1978) Upstone (1979)
	Monochaetia mali	Naik (1979)
	Clathridium corticola	Thind et al (1979)
	Penicillium expansum Link P. divergum P. cyclopium Westling P. spinulosum Thom	Boreeka (1979)
	Corticium centrifugum	Weresub & Illman (1980)
	Fusarium solani (Mart) App & Wollenw F. equiseti (Corda) Sacc	Sumbali et al (1980)
	Penicillium expansum Link Stemphylium botryosum (Pleospora herbarum) Wallr	Babovic et al (1980)

1	2	3
	Fusarium lectis Monilia (Sclerotinia) fructigena Gleosporium fructicenum (Glomerella cingulata)	Babovic et al (1980)
	Calcariosporium sp Rhizopus oryzae Went & Goerlings Trichothecium roseum Link	Garcha & Singh (1980)
	Penicillium expansum Link Cladosporium herbarum (Pers) Link	Eicho (1981)
	Gleosporium sp Botrytis cinerea Pers Penicillium sp Nectria gallicena Berk. Bark	John & Burth (1981)
	Botryodiplodia theobromae Pat	Bhargava et al (1981)
Mangifera indica	Physalospora rhodina (B &C)	
	Hendersonia creberrima	Brodrick & Wosthuisen (1977)
	Phomopsis mangifera Ahmad	Laxminarayana & Reddy (1978)
	Aspergillus niger van Tiegh As. terrus As. flavus As. fumigatus Fres Botryodiplodia theobromae Pat Botryosphaeria ribis	Garcha & Singh (1980)

1	2	3
	A. flavus Link	Majumdar & Modi (1981)
Musa (Banana)	Thielaviopsis paradoxa (De seynes)	Garcia (1972)
	Botryodiplodia sp	Lantican & Quimio (1978)
	Fusarium moniliforme Sheldon	Khanna & Chandra (1979)
	F. roseum Link	
	Fusarium moniliforme (Gibberella fujikuroi) Sheldon	Abdel-Sattar et al (1979)
	Ceratocystis paradoxa (de Seynes) Moreau	Abdel-Sattar et al (1979)
	Cylindrocarpon (Nectria) radicicola	Sharma & Khan (1981)
	Aspergillus terrus Thom	
Phyllanthus emblica L. (Aonla)	Phoma emblicae Jamaluddin	Jamaluddin et al(1979)
	P. putaminum Speg	Pandey et al (1980)
	Cladosporium tenuissima Cooke	Jamaluddin (1981)
	C. cladosporioides (Fres) de vries	
Physalis peruviana	Fusarium equiseti	Rao & Subramaniam (1979a)
Cape gooseberry or Rasberry	Drechslera (Setosphaeria) rostrata	Tandon & Singh (1981b)
	Alternaria alternata (Fr) Keissler	
	Curvularia lunata (Cochlibolus lunatus) var-aeria	
Prunus avium (Cherry)	Alternaria dendritia	Gupta & Verma (1980)
	Trichothecium sp	Bhargava et al (1981)
	Rhizopus sp	
	Alternaria sp	
	Fusarium sp	

1	2	3
P. domestica (Plum)	Monilinia fructicola (Wint) Honey	Kable (1973)
	Sclerotinia fructicola	Tate & Corbin (1979)
	Glomerella cingulata	Maiti et al (1981)
P. persica (Peach)	Phytophthora syringae (Kleb) Kleb	D' ercole & Flori (1978)
	Sclerotinia fructicola	Tate & Corbin (1979)
	Monilia fructicola (Wint)	Phillips & Harris (1980)
Psidium guajava (Guava)	Macrophoma allahabadensis	Kapoor & Tandon (1970)
	Diplodia natalensis	Rajagopalan & Wilson (1972)
	Mucor heimalis Wehmeyer	Kunimoto et al (1977)
	Phytophthora nicotianae var Parasitica	Singh et al (1978)
	Pestalotia olivacea Guba	Dhingra & Mehrotra (1980)
	Aspergillus nidulans var	Garcha & Singh (1980)
	Rhizopus stolonifer (Ehrqnb ex Fr)	Ooka (1981)
Punica granatum L. (Pomegranate)	Aspergillus flavus var	Srivastava & Tandon (1971)
	Coniella novae - zelandiae	Sharma & Jain (1979)
	C. granati	
	Aspergillus niger van Tiegh	Philip (1981)
Pyrus Communis L. (Pear)	Coremiella cubispora	Lucas et al (1977)
	Sclerotinia fructigena	Byrde & Willetts (1977)
	S. fructicola	
	S. laxa	
	S. laxa f. sp. mali	

1	2	
	Sclerotium rolfsii Sacc	Sumbali & Mehrotra (1980)
	Trichoderma viride Pers Ex Fr	Roy & Sharma (1980)
	Penicillium crustosum	Garcha & Singh (1980)
	Phoma glomerata (Corda) Wr & Hochapfel	Chohan & Chand (1981)
	Penicillium expansum Link P. diversum P. cyclopium Westling P. spinulosum Thom	Boreeka (1979)
Vitis vinifera L. (Grape)	Botrytis cinerea Pers	Macclellan & Hewitt (1973)
	Greeneria fuliginea Scribner & Viala	Prakash et al (1976)
	Guignardia bidwellii (Ellis) Viala & Ravez	Perrin & Ramsdell (1978)
Zizyphus mauritiana L. (Ber)	Phoma hissarensis Alternaria state of Pleospora Colletotrichum gloeosporioides Trichothecium roseum	Gupta & Madan (1979)
	Alternaria alternata (Fr) Keissler Fusarium decemcellulare (Calonectria rigidiuscula) Cladosporium cladosporioides (Fres) de vries	Wadia & Manoharachry (1981)
Allium cepa (Onion)	Sclerotium cepivorum Berk	Rondomanski (1978)
	Aspergillus niger van Tiegh Cephalosporium (Acremonium) curtipes	Sharma & Roy

1	2	3
	Pleospora herbarum	Wu (1978)
	Sclerotinia sclerotiorum	Tsupkova (1979)
	Aspergillus niger van Tiegh	Raju & Raj (1981)
Allium sativum L. (Garlic)	Fusarium solani (Mart) App et Wr emend	Mic ail et al (1972)
	Cephalosporium (Acremonium) curtipes	Roy et al (1978)
	Penicillium genum P. corymbiferum	Georgieva & Kotov (1979 b)
	Fusarium oxysporum F. solani	Wu (1979)
	Fusarium oxysporum f. sp capae Penicillium genum P. corymbiferum Helminthosporium allii	Georgieva & Kotov (1979 c)
Amorphophallus campanulatus Blume	Sclerotium rolfsii Sacc	Tyagi & Sharma (1978)
Benincasa hispida (Petha fruit)	Fusarium solani F. moniliforme (Gibberella fujikuroi)	Sharma et al (1981)
	Penicillium citrinum	Roy et al (1981)
Capsicum sp	Colletotrichum capsici	Singh et al (1978)
	Alternaria alternata (Fr) Keissler	Shawkat et al (1979)
	Fusarium solani F. oxysporum	Nicosa & Xiag (1979)

1	2	3
	Helminthosporium (Drechslera) halodes	Laxminarayan & Reddy (1980)
	Myrothecium roridum	
	Pestalotiopsis theae	
	Aspergillus sp	Seenappa et al (1981)
Cucumis sp	Pythium butleri	Nasfy et al (1978)
	A sterile phycomycete	
	Cladosporium herbarum	Georgieva & Kotov (1979 a)
	Penicillium italicum	
Daucus carota	Alternaria radicina	Varkhuseheva (1973)
	Stemphylium botryosum	
	Chalaropsis thielaviodes	Schaffrath (1978)
	Sclerotinia sclerotiorum	Tsupkova (1979)
	Phytophthora sp	Stelfox & Henry (1979)
	Stemphylium radicinum (Alternaria radicina)	Glaser (1979)
	Geotrichum candidum	Suhag & Duhan (1981)
Lycopersicum esculentum (Tomato)	Pythium ultimum	Pearson & Hall (1974)
	Pa aphanidermatum	Sonoda (1974)
	Geotrichum candidum	Barts (1981)
	Rhizoctonia solani	
	Sclerotium rolfsii	
	Pythium aphanidermatum	
	Botryodiplodia theobromae	Onesirosan & Fatunla (1977)
	Fusarium equiseti	
	Fa xylarioides	

1	2	3
	Synecephalastrum racemosum	Rao & Subramanian (1979b)
	Fusarium solani (Mart) Sacc	Garg & Gupta (1979)
	Alternaria alternata (Fr) Keissler	Tani et al (1979)
	Phoma destructiva Ascochyta (Didymella) lycopersici	Kogan (1979)
	Nitrospora oxyzae (Berk & Br) Poteh Stemphylium vesicarium (Wallroth) Simmor	Chary et al (1980)
	Phytophthora nicotianae var nicotianae	D' ercole (1981)
	Myrothecium cermicheeli	Tandon & Singh(1981 a)
Momordica Charatia (Bitter gourd)	Alternaria tenuissima Myrothecium roridum	Sharma & Bhargava (1979)
	Glomerella cingulata	Verma et al (1981)
Solanum tuberosum	Phytophthora erythroseptica Pethybr	Carroll & Sasser (1974) Lopkova (1976)
	Ophiobolus porphyrogonus (Tode) Sacc	Popkova & Kovaleva (1974)
	Acremoniella atra (Corda) Sacc	El-Khadem & El-Kazzaz (1978)
	Fusarium solani (Mart) Sacc	Braslavska (1978)
	Aspergillus flavus Link	Gupta et al (1978)
	Fusarium coeruleum (Lib) Sacc	Langerfeld (1979)
	Phoma solanicola Prill & Dell	Popov (1979)
	Fusarium trichothecioides Woll	Marte & Tamburi (1980)

1	2	3
	Fusarium equiseti (Corda) Sacc	Rai (1980)
	Phoma cupyrena Sacc	Langerfeld (1980)
	Phoma spp	Janke & Zott (1981)
	Fusarium sp	Gusev (1981)
Solanum melongena L.	Phomopsis vexans (Sacc et Syed) Harter	Panwar et al (1972) Chowdhury & Hasina (1981)
	Chrysosporium pyrinosum (Gilman & Abbott)	Dhingra & Mehrotra (1980)
	Rhizopus nodosus	Vyas et al (1980)
	Fusarium moniliforme Sheld	Datar (1981)
	Rhizopus oryzae Went & Geerlings	Ali & Shukla (1981)
Trichosanthus anguina (Snake Gourd)	Penicillium janthinellum	Rao & Thirupathaiah (1980)
I. dioica Roxb (Pointed gourd)	Fusarium equiseti (Corda) Sacc Alternaria alternata (Fr) Keissler Glomerella cingulata (Stoneem) Spauld & Schrenk Helminthosporium sp Curvularia lunata (Walker) Deodijn var aeria (Batista Lima, & Vasconcelos) Ellis	Kritapyan & Singh (1980)
Zingiber officinale (Ginger)	Diplodia natalensis	Wilson & Balagopal (1973)
	Pythium deliense	Bawaro & Joshi (1976)

BIOCHEMICAL CHANGES:-

Fungal invasion brings about changes in chemical constituents of the fruits. There is a great shift in the concentration of carbohydrates, organic acids, phenols, aminoacids, vitamins etc. (Don'ze 1964, Srivastava & Tandon 1966, Stretch & Cappellini 1965, Aulakh et al 1970a, 1970b).

Carbohydrates:- Drastic changes in sugar content has been observed in apples infected with Aspergillus terreus Thom (Chandra & Tandon, 1963), guava, sapodilla, papaya and banana fruits with Pestaloti psidii P. Henn, Colletotrichum papayae P. Henn, Fusarium sp, Phoma psidii, Gleosporium sp, Botryodiplodia theobromae Pat, and Pestalotia sapodilla P. Henn (Ghosh et al 1964) cucumber by Pythium aphanidermatum (Edn) Fitz (Mc Comb & Winstead, 1964).

Ghosh & Tandon (1965a) reported the presence of oligosaccharides in mango fruits infected with Collectrotrichum gloeosporioides Penz; in musambi infected with Botryodiplodia theobromae Pat. Srivastava & Tandon (1966) reported that oligosaccharides developed on the 4th day after infection. Oligosaccharides were also detected as a result of infection with Pestalotia sapota P. Henn & B. theobromae Pat in sapota & with Gleosporium psidii Delacr (L. Fr) on a variety of guava (safeda). However, infection of Pestalotia psidii P. Henn & Phoma psidii on guava did not form oligosaccharides (Tandon, 1967).

Banana infected with Botryodiplodia theobromae Pat showed loss in fructose and glucose content (Williamson & Tandon, 1965). Kapoor & Tandon (1967) reported decrease in glucose, fructose and sucrose content in guava fruits infected with Macrophomina allahabadensis Kapoor & Tandon, and musambi fruits infected with Aspergillus niger van Tiegh (Singh, 1968). Similar results have been observed in a tomato variety infected with Drechslera australiense (Bugn) Subram & Jain (Kapoor, 1968); papaya and banana fruits infected with Rhizopus stolonifer (Fr) Lind, tomato fruits infected with Phoma destructiva (Aulakh et al, 1970b).

Chahal & Grover (1972) while carrying out changes in the sugar content in Chilli fruits infected with Choanephora cucurbitarum (Berx & Rav) Thaxter, found that in the healthy fruits two sugars viz. fructose, D-xylose were detected but after 3 days of pathogenesis both the sugars disappeared. Changes in sugar content have also been reported in banana fruits infected with Alternaria alternata (Fr) Keissler & Cochliobolus [illegible] spicifer Nelson (Prasad, 1974). tomato fruits infected with Cylindrocarpon scoparium Morgan, Colletotrichum sp Myrothecium roridum Tode ex Pers, Chilli fruits infected with Rhizopus stolonifer (Fr) Lind (Tandon et al, 1974), that in papaya infected with Phomopsis caricae papayae Petrax & Cif (Dhingra & Khare, 1975); tomato infected by Alternaria solani (Ellis & Mart) Jones & Grout & A. tenuis Nees ex Pers (Mehta et al, 1975) and musambi fruits infected with Botryodiplodia theobromae Pat (Ali, 1976). Singh & Chohan (1981) reported that the concentra-

-tion of 3-4 sugars which were detected in healthy fruits of cucurbits decreased when infected with Macrophomina phaseolina.

Organic acids:- Organic acids like fumaric, tartaric, malic, succinic, oxalic acids which are the chief constituents of several fruits and vegetables also undergo changes as a result of infection with pathogens. Kapoor & Tandon (1969), Singh & Tandon (1970) Tandon, Srivastava & Jamaluddin (1974) reported that organic contents of fruits decreased in infections with various fungi.

Fumaric, malonic and citric acids which were observed in healthy fruits of tomato, were also observed in fruits infected with Drechslera australiense (Bugn) Subram & Jain, but the content was very low except that of fumaric acid which showed an increase in the content. The production of succinic acid during pathogenesis of fruits is interesting (Kapoor & Tandon 1969). Tandon et al (1974) reported that chilli fruits infected with Rhizopus Stolonifer (Fr) Lind, & tomato fruits infected with Colletotrichum sp, Cylindrocarpon scoporium Morgan, Myrothecium roridum Tode ex Pers showed a decrease in the organic acid content. Mehta et al (1977) carried out analysis of extracts of healthy and diseased tomato fruits infected with Alternaria solani (Ellis & Mart) Jones & Grout & A. tenuis Nees ex Pers & observed that there was considerable changes in organic acid content due to

pathogenesis. These observations led them to conclude that succinic acid was synthesized in the diseased fruits. Jamaluddin (1979) while studying changes in the organic acid content of healthy and diseased fruits of peach (Prunus persica) found that in the fruits infected with Aspergillus flavus the quantity of succinic, malonic and citric acids increased while the quantity of malic and tartaric acid decreased. Fumaric acid and an unknown acid with (Rf 0.09) were detected only in the diseased fruits. In general the total amount of organic acids increased under pathogenesis.

Recently Majumder & Modi (1981) while carrying out biochemical studies on spoilage of mangoes by Aspergillus flavus Link observed a high level of citric acid in infected fruits. Thind et al (1979) reported that the concentration of the 10 organic acids which were found in high quantity in healthy fruits showed a slight decrease in quantity after infection with Clathridium corticola in apple fruits.

Ascorbic Acid:- It has been observed that the ascorbic acid content decreases when fruits are infected with fungal pathogens (Bhutani 1946).

Mango fruits infected with Colletotrichum gloeosporioides Penz & Aspergillus niger van Tiegh, and guava fruits infected with Pestalotia psidii P. Henn, Phoma psidii P. Henn & Gloeosporium psidii P. Henn, (Ghosh et al 1965) showed decrease in ascorbic acid content. The rate of decrease of ascorbic acid was faster in mango fruits

infected with Botryodiplodia theobromae Pat (Srivastava & Tandon, 1966a). In musambi fruits which were infected with B. theobromae Pat no ascorbic acid was detected (Srivastava & Tandon 1966a). Chahal & Grover (1972) carried out studies on chilli fruits infected with Choanephora cucurbitarum (Berk & Rav) Thaxter; and reported that ascorbic acid content of diseased chilli fruits declined rapidly after 5 days of pathogenesis.

Significant losses in ascorbic acid content was also observed in papaya fruits damaged by 6 Post harvest pathogens, namely Alternaria tenuis (A. alternata) Nees ex Pers, Chaetomium globosum Kunze & Sohm, Curvularia lunata (Coehliobolus lunatus) (Walker) Boedij, Cylindrocarpon tonkinense, Fusarium oxysporium (Schl ex Fr) Synd et Hans & Helminthosporium spiciferuim (Coehliobolus spicifer) (Prasad & Verma, 1978). Agrawal & Bisen (1978) reported that when Aspergillus niger van Teigh & Alternaria tenuis (A. alternata) Nees ex Pers isolated from apple were inoculated into 5 apple cultivars, decrease in ascorbic acid was seen and this was much faster the apples inoculated with Aspergillus niger van Teigh than Alternaria alternata Nees ex Pers. Fruits of litchi infected with Fusarium semitectum Berk & Rav, Alternaria alternata (Fr) Keissler & Cylindrocarpon tonkinense reduced the ascorbic acid content by 87.4 - 90.7% although some loss was also observed in control fruits which may be due to over ripening (Prasad & Sinha 1979). Decrease in ascorbic acid content during pathogensis of lemon fruits with Fusarium oxysporum was

reported by Narwria & Reddy (1980).

Phenols:- Phenolic compounds are also one of the important constituents of a plant cell. These compounds provide resistance in fruits and vegetables. Higher concentration of phenolics in a variety of tomato resistant to Alternaria solani (Ellis & Mart) Jones & Grout was reported by Bhatia et al (1972). Similarly in tomato fruits infected with Alternaria tenuis Nees ex Pers & A. solani (Ellis & Mart) Jones & Grout were found to contain higher amount of phenols, (Mehta et al 1975).

A phenolic substance (RF value .32) was detected in the healthy fruits which decreased during pathogenesis by Alternaria solani and was totally lost in 12 days old infections. Two phenols (RF. .3 & .15) were observed in healthy host tissue of apple fruits but after infection with Clathridium corticola, the phenol (RF. .3) increased (Thind et al 1979). Post infection biochemical changes in apple fruits rotted by Penicillium expansum Link, Glomerella cingulata Spauld & Schen Trichothecium roseum Link, Monilinia (Sclerotinia) laxa & Rhizopus stolonifer (Fr) indicated a reduction in the phenolic content (Kaul & Munjal 1981).

Amino acids:- Post infection changes in amino acids have been studied in seasonal fruits such as in melons infected with Colletotrichum lagenarium (Pass) Ell & Hver (Tonze, 1964); Papaya with Gloeosporium papayae P. Henn, Botryodiplodia

theobromae Pat, Colletotrichum capsyae P. Henn, Rhizopus nigricans Ehrenb; guava fruits with B. theobromae Pat, Phoma psidii P. Henn, Cleosporium psidii P. Henn, Pestalotia psidii P. Henn (Tandon, 1967); banana fruits with B. theobromae Pat, Cleosporium musarum Cooke & Mass.

Mango, sapota and citrus fruits infected with B. theobromae Pat, tomato with Phoma destructiva Plower (Aulakh et al 1970a) showed significant changes in the amino acid content. Chahal & Grover (1972) carried out studies on healthy chilli fruits and also fruits infected with Choanephora cucurbitarum (Berk & Rav) Thaxter. They detected eight amino acids namely Aspartic acid, Glutamic acid, L-Alanine, B-Alanine, tryptophane, Arginine, Histidine, Glycine in healthy fruits but during pathogenesis all except glycine were reduced considerably. Tomato fruits infected with Cylindrocarpon scoparium Morgan, Myrothecium roridum Tode ex Fer, Colletotrichum sp, Alternaria tenuis Nees ex Pers, A. solani (Ellis & Mart) Jones & Grout (Mehta et al 1975 a,b) also exhibited a sharp decline in the amino acid content during pathogenesis.

Mehta et al (1977) carried out analysis of extracts of healthy and diseased tomato fruits infected with Alternaria solani (Ellis & Mart) Jones & Grout & A. tenuis Nees ex Pers & observed changes in the composition of aminoacids. The content of free aminoacids was considerably altered qualitatively and five aminoacids appeared in the diseased fruits. Singh & Chohan (1979) carried out experi-

-ments on changes in free aminoacids in fruits of bottlegourd, muskmelon & watermelon due to infection of Pythium butleri. Thind et al (1979) observed post infection changes in amino acid contents in apple fruits rotted with Clethridium corticola. Of the 10 free amino acids detected in the healthy tissues of apple fruits histidine, arginine, aspatic acid, alanine, leucine, valine, phenylalanine were completely utilized or disintegrated by the pathogen during pathogenesis. However, three newly synthesized aminoacids viz. Cystine, aminobutrytic acid and tyrosine were detected in the infected tissues. Loss in amino acid content was also observed in tomato fruits infected by Phoma destructiva Plower (Hasija & Batra, 1981).

Kaul & Munjal (1981) determined biochemical changes including changes in aminoacids in apple fruits inoculated with Penicillium expansum Link, Monilia (Sclerotinia) laxa & Rhizopus stolonifer (Fr) Lind and obtained very interesting results.

Factors affecting post harvest losses:- Large number of factors have been found to influence these losses in fruits and vegetables during storage and transits. Broadly they are classified as physical, chemical and biological factors.

Physical:- This category includes (a) the initial quality of the commodity (b) the temperature at which the product is

held during handling, storage, transport and distribution (c) the R. H. of the post harvest environment (d) the use of controlled or modified atmospheres during storage and transit (e) chemical treatments for the control of decay (f) heat treatments for decay control (g) packing and handling systems.

Initial quality:- Fruit harvested at an immature stage may never ripen properly and may consequently constitute a post harvest loss when rejected at the retail or consumer level. Similarly fruits harvested at an over-mature stage may not survive the handling to which it is subjected during marketing. Thus these fruits become a loss in the marketing sequence (Harvey 1978).

Harvey (1978) further pointed out that the initial quality of fruits should be free from infections to avoid further rotting during storage and transport. This post harvest loss can be reduced by adopting an integrated approach of control methods (Harvey, 1978).

Temperature:- Normally for keeping the fruits and vegetables fresh, the storage is done at the temperature - in cold storage and in refrigerators. Parpia (1976) pointed out that lack of refrigeration capability is largely responsible for major post harvest losses in many developing countries. On the basis of experimentation, compilations have been made to indicate the optimum temperatures for maintenance of quality and prevention of losses during storage and transit.

(Asby, 1970, Ashare, 1974, Lutz & Hardenbury 1968, Pantastico 1974, Redit 1969, Whiteman 1957). Mac Leod et al (1976) pointed out that there is a relationship between post harvest losses and temperature. These authors reported that lettuce become unsalable in only 12 days at 15°C but not until 35 days at 0°C, Asparagus was found more perishable as it become unsalable in only 5 days at 15°C but not until 35 days at 2°C. Storage of asparagus at 0°C is not permissible as it brings about chilling injury.

Lutz & Hardenbury (1968) showed that many tropical fruits such as mangoes and bananas are damaged if stored at a temperature below 7.5°C. Similarly green vegetables are also susceptible to freeze injury and are, therefore, recovered for storage at temperature above 7.5°C.

Temperature during storage has another problem of control of microorganisms. Certain microorganisims thrive best and are capable of growing even at low temperatures.

Studies on 11species of Rhizopus, which is a very important pathogen of fruits and vegetables, were carried out by Barter & Weimer (1922) to determine the optimum temperature required by the species to grow. Rhizopus nutgarpi Kacib, R. nigricans Ehrenb, grow best at 20-25°C. R. Oryzae Fischer & R. nodusus Namysolwski required 30°C as optimum temperature. R. Chlonsis Ehrenb developed at 35°C.

Karmakar & Joshi (1940), Sethi (1948) and Mukerjee (1961) reported that below 10°C pathogens failed to grow on mango. A temperature range of 20 - 25°C was found to be the optimum temperature for the growth of Alternaria tenuis Nees ex Pers on Pears (Tandon & Ghosh 1962).

Tandon (1967) reported that Gleosporium musarum Cke & Mass caused least decay on banana fruits when stored at 10°C. Pestalotia sapotae P. Henn & Botryodiplodia theobromae Pat could not cause any decay on sapodilla at 10 - 15°C but considerable losses were observed at temperature between 25 - 30°C (Tandon & Mishra, 1969). Similar studies to determine the optimum temperature for pathogens to grow were made on Litchi fruits infected with Aspergillus flavus Link, A. niger van Tiegh, A. quadritientus Thom & Raper, A. Variecolor Thom & Raper, A. nidulens (Eidam) Wint, Cylindrocarpon tankinese Bunai, Pestalotia sp & Colletotrichum gloeosporioides Penz which caused optimum rot at temperatures between 15 -25°C.

Mehta et al (1977) while carrying out pathological studies on fruit rot of tomato caused by Alternaria solani (Ellis & Mart) Jones & Grout & A. tenuis Nees ex Pers reported that the maximum rotting occured at 28°C. Alternaria sp., causing a fruit rot of Juglans regia failed to germinate at 45°C, with 30°C as the optimum temperature for germination (Verma et al 1977). Colletotrichum capsici (Syd) But & Bis causing fruit rot of chillies grew best at 30°C (Singh et al 1978). Potato

decay by Fusarium solani (Mart) App & Wall F. roseum Link & F. oxysporum (Schl ex Fr) Synd et Hans was minimum when tubers were stored at 19°C (Lee 1978). Sharma & Khan (1979) reported that optimum temperatures for the growth of Alternaria alternata (Fr) Keissler & Cladosporium cladosporoides on cape gooseberry were 20 & 25°C respectively. Fusarium solani (Mart) App & Wall & F. oxysporum (Schl ex Fr) Synd et Hans causing fruit rot of chillies grew and sporulated at 20 - 30°C. (Micosa & Ilag, 1979). Blue mold on garlic in Bulgaria caused by Penicillium canum & P. corymbiferum developed best at 20 - 25°C and 25 - 30°C respectively (Georgieva & Kotev, 1979). Low temperature (5,10,15°C) were unfavourable for the initiation of fruit rot in lime fruits caused by Aspergillus niger Van Teigh while at 25,30,35°C rapid rot occurred within a few days (Khanna & Chandra 1979 a). Fusarium moniliformae (Gibberlla fujikuroi) Sheldan & F. roseum Link causing banana fruit rot was maximum at 25 - 35°C (Khanna & Chandra 1979 b). Rot of cucumber fruits during transportation caused by Cladosporium herbarium (Pers) Link & Penicillium italicum developed at low temperature 8 -10°C maintained in refrigeratfed cars (Georgieva & Kotev 1979).

Cylindrocladium clavatum causing potato tuber rot was less at 15°C and 20°C, with no measurable disease occurring at 10°C. (Bolkan et al, 1981). Bhargava et al (1981) carried out studies on Botrydiplodia rot of apple and reported maximum rot at 36°C and minimum at 20°C while no rotting

was observed at 10°C. Pathological studies regarding optimum temperature for the development of Phomopsis vexans (Sacc & Syd) causing rot of brinjal fruit carried out by Choudhary & Pasija (1981) indicated that 25°C was the optimum temperature for rotting.

Relative humidity:- Like temperature relative humidity equally plays an important role in the development of post harvest diseases. It not only affects the moisture loss from fruits or vegetables as evidenced by shriveling or wilting, but also affects the activity of the decay causing organisms. Moisture loss is serious in commodities that are stored for a long time e.g. apples etc. or in those that may be stored only for short periods.

Fungi in general required high moisture level for their growth and metabolism. Generally 95 to 100 percent relative humidity supports highest growth of most of the fungi. Relative humidity below 80 to 85 percent are by and large inhibitory for them. Conidia of Monilinia fructicola (Wint) Honey and survive till a very long period at 15 percent RH (Naqvi and Good 1957).

Very high relative humidity generally favour the growth of organisms causing decay, but do not necessarily result in increased decay losses in fruits and vegetables (Harvey, 1978). In potatoes for example, high RH promotes curing and healing by suberization which reduces invasion by micro organisms (Artschwager, 1927).

Recent studies on the effect of RH during storage of Brussels sprouts, cabbage, leeks showed that at low temperature there is less decay at 98 - 100 percent relative humidity than at 90 - 95 percent or lower. (Lentz & van den Berg 1971, Pendergrass & Isenberg 1974). The rot of banana, apple, mango, tomato and potato by Rhizopus spp. was not very much influenced by RH. However, a RH of 50 percent or above allowed the rapid development of soft rot with moderate to heavy intensity (Thakur 1972).

Mehta et al (1977) reported 100 percent as the optimum relative humidity for the growth of Alternaria solani (Ellis & Hart) Jones & Grout & A. tenuis Nees ex Pers on tomato fruits. Khanna & Chandra (1979) reported that fruits of lime when stored at 90 - 100 percent RH showed 100 percent rot in nine days due to infection caused by Aspergillus niger Van Tiegh. Maximum rotting of banana due to Fusarium moniliforme (Gibberella fujikuroi) Sheldon & F. roseum Link (Tandon & Chandra 1979) occurred when the relative humidity was 90 percent. High intensity of fruit rot of Pyrus communis due to Trichoderma viride occurred at 60 percent RH (Roy & Sharma, 1980). Potato tubers stored in polyethylene boxes were badly damaged by Fusarium sulphureum (Gibberella Cyanogena) at higher RH (98 - 100 percent compared with 80 percent) (Langerfield, 1981). Maximum rotting of brinjal fruits due to Phomopsis vexans was seen at 55 percent RH (Choudhary & Nasija, 1981).

High relative humidity, when desired in storage is generally maintained by the use of a refrigeration system. Steam injectors or atomizers have also been used for increasing RH in storage (Gentry & Guillon, 1966). Packing of fruits with protective films creates an environment with high RH within the package and thus makes the print liable to more damage than in storage rooms (Hardenburg, 1971, 1973).

Controlled or modified atmospheres:- Controlled or modified atmospheres are equally essential for good storage of fruits. These conditions are to be supplemented with refrigeration to have a better effect (Dewey 1977). Dewey et al (1969) Mannis et al (1971) studied the effect of controlled atmospheres for the storage and transport of perishable agricultural commodities. By and large, the controlled and modified atmospheres inhibit the activity of decay organisms. Griverson (1969) reported that fruits which undergo ripening after harvest are more sensitive to controlled atmospheres. Therefore, controlled atmospheres are effective for those fruits that cannot be stored at the optimum temperature.

Lettuce was found to be stored better at 0°C and is therefore usually transported at temperature of 4°C to 5°C. Reduction of the amount of O_2 in a rail, car or truck from a normal 21 percent to 2 to 5 percent slows down the respiration rate (Linton, 1967 and Morris & Lader, 1977).

Strawberries shipped by air are much above their optimum holding temperature of 0°C for the major part of the transit period (Harvey et al, 1960). Therefore maintenance

There was a reduction in decay of peach rots due to Monilia sp, Botrytis sp and Rhizopus sp by post harvest treatment with thiabendazole carbondazin, imazalil, iprodione, captafol & vinclozolin (Nombeix et al 1980). White rot disease of garlic caused by Sclerotium cepivorum Berk was reduced when fungicides like vingozolin, iprodione, benomyl & dicloran was applied as a bulb dust (Tamiotti 1981).

Heat treatments- Heat treatment has been widely recommended because it controls organisms that have already penetrated the fruit as well as those located on the surface of the fruits. In a way this treatment is comparable to pasteurization of dairy products in that the temperatures and exposure times do not cause appreciable change in the texture, flavour or other characterstics of the product, but do inactivate the pathogens. Studies carried out by several workers have shown that care should be taken to control the temperature because high temperature may cause injury to the fruit. Papaya and peaches have been treated with heat treatments to control fruitfly and pathogens such as Colletotrichum in Hawaii. Treating peaches for 3 - 5 minutes in water at 49°C or 1.5 minutes at 54°C controlled both brown rot (Monilinia fructicola) and Rhizopus rot (Rhizopus stolonifer) (Smith et al 1964). A 2 to 3 minutes exposure to 52°C in water was most effective for control of fruits decay, without causing injury (Smith & Redit, 1968). Akamine and Arisumi (1953) and Akamine and Goo (1969) suggested a treatment of

papaya for 20 minutes at 49°C for controlling anthracnose and other diseases. Akamine (1977) also suggested a 20 min dip in hot water at 47°C for controlling decay of mangoes. Wells (1971) recommended 1.5 min dips in water at 52°C for reducing the decay of nectarines by 65 to 75% compared to decay of untreated fruits. However, when hot water treatment was supplemented with treatment with Captan, benomyl, the effectiveness increased. This combination has another advantage that a very low concentration of the chemical was required as that when used alone. Spalding & Reeder (1980) showed that post harvest decay was controlled by dipping mango fruits in hot water (52°C) containing .1% benomyl for 1-3 min.

Packing & Handling Systems:- Improper packaging and handling systems also aggravate the losses due to post harvest disease. Proper packaging of a product prevents not only bruising and crushing but can also reduce the moisture loss and pilferage in addition to maintaining a desirable environment during marketing.

According to Perkins (1959) packing of apples in polyethylene bags can reduce bruising caused during transport to the extent of 50%. Bags should be placed on their sides in layers within a partitioned master container rather than placing them upright through the full depth of the container.

Tight full packing of pears, plums, apricots, cherries and other tree fruits had resulted in reduction of losses from bruising. This kind of packing, however, would be different for different kinds of fruits and separate studies are needed for each type of fruits and vegetables. Fruits like cherries, peaches which are soft skinned may require some extra padding in packing (Gentry et al, 1965, Mitchell et al 1964, 1965, 1967).

According to Guillev (1960), Olson et al (1960), Patchen (1969), package design must be coordinated with cooling systems and refrigeration practices with in storage and in the transit taking into consideration the strength of the packing.

Box liners for cherries have been found effective in reducing the blue mold rot caused by Penicillium expansum LK ex Thom. Mc Intosh and Porritt (1958), proved that box liners caused an increase in decay because of the abundant moisture with in the packed boxes e.g. Bull's eye rot caused by Neofabraea prennans Kieh was much higher in fruit stored in box liners than in those stored without liners. Hardenburg (1974) showed that treatment of fruits with certain fungicides before packing considerably reduced losses from decay. Box liners used for packing citrus when treated with biphenyl reduced the damage of citrus fruits from green mold rot caused by Penicillium digitatum. Saco. and blue mold

rot caused by Pa italicum Wolmer (Ryan et al. 1963). According to Gerrhault (1955) the storage life of pears can be extended by 6 to 8 weeks with reduction in moisture loss by packing the fruit in sealed polythylene liners. Singh & Gupta (1980) proved that decay of citrus fruits could be reduced when the fruits are stored in gunny bags or wooden boxes rather than, when stored in bamboo baskets.

Both grocers and consumers are responsible for reducing the post-harvest losses in perishable commodities. In addition to this it also involves each and every individual who handles the product during marketing.

It is, therefore, necessary to know the factors which influence the post harvest losses in fruits while they are stored in the consumbers' place. Practically no work has been done on this aspect, more particularly in India.

PUBLIC PLAN OF WORK

FUTURE PLAN OF WORK

It is clear from the brief review presented here that despite large number of studies there are lacunae in our knowledge which need to be filled in. Moreover nothing precisely is known what precautions to be taken to minimise the losses due to diseases in transit. Therefore, with this aim in view it is proposed to study the following:-

1. To isolate fungal pathogens from diseased fruits and vegetables viz. mango, apple, peach, banana, citrus, tomato, brinzal, potato, capsicum, carrot etc. and to diagnose them upto specific level.

2. To test their pathogenicity in fruits in the laboratory.

3. To study the effect of different types of semi solid and liquid media, different carbon and nitrogen sources and mineral elements on the growth of some of the known pathogenic fungi.

4. To study the effect of temperature, relative humidity and P^H on the development of pathogens isolated in 1 above.

5. To study the post-infection changes in the phenolic, aminoacid, carbohydrates, organic acid and ascorbic acid contents in diseased as well as healthy fruits and vegetables.

6. Effect of various substances on the production of enzymes.

7. To determine the effect of different packing systems on the development of post harvest losses.

8. To devise control measures.

MATERIALS AND METHODS

MATERIALS AND METHODS

Survey & Collection of diseased material:-

Diseased fruits and vegetables will be collected from the market and will be brought to the laboratory for isolating the fungi associated with the rot and identifying them.

Isolation, purification & maintenance of cultures:-

The diseased fruits and vegetables which will be collected from the local market will be kept in a moist chamber at 20 - 28°C, to facilitate the growth and sporulation of fungi.

For isolation of pathogens, the diseased fruits will be thoroughly washed with tap water and then the surface will be sterilized with .1% $HgCl_2$ solution by keeping them into the solution for 1 to 2 minutes. After repeated washing with distilled H_2O, diseased lesions will be cut from the junction of diseased tissues with the help of sharp sterilized knife. The diseased tissue will be transferred in sterilized petridishes containing PDA. Before transference of the diseased tissue the petridishes will be incubated at 28 $\pm$ 1°C. Then the fungal hyphae growing out of the diseased tissues will be picked and transferred to fresh petri dishes containing PDA. The purified isolates will be maintained on PDA slants at lower temperatures. The isolates will be identified with the help

of available literature.

Pathogenicity tests-

Pathogenicity will be performed to confirm the pathogenic ability of the isolated pathogens. Uninjured, healthy specimens will be surface sterlized with .1% $HgCl_2$ solution and will be thoroughly washed with distilled water. The specimen will then be inoculated either by pricking, or by making a hole with the help of a sterlised cork borer (Granger and Horne, 1924). In both the cases the inoculum consisting of a mycelial agar disc will be cut from the margin of the freshly grown colony and will be placed over the incision made earlier. The inoculated specimen will be kept in moist chambers at 28 $\pm$ 1°C. Uninoculated fruits will also be kept in the same conditions which will serve as control. Fruits will be observed daily and the symptoms will be compared with the original disease symptoms. Again the pathogens will be isolated and will be compared with the original cultures. Isolation and inoculation will be repeated many times so as to confirm Koch's postulates.

Effect of different Culture media-

After identifying the fungi, the pathogen shall be cultured on different media, to find out the most suitable basal medium for further studies.

a) Potato - dextrose - agar medium:-

Peeled potatoes	200 g
Dextrose	20 g
Agar	20 g
Distilled Water	1000 Ml.

b) Martin's Rose Bengal Streptomycin agar medium:-

Dextrose	10 g
Peptone	5 g
Potassium dihydrogen phosphate ..	1 g
Magnesium sulphate	.5 g
Rose Bengal (1 part in 30,000 parts of the medium)	
Agar	20 g
Streptomycin	.03 g
Distilled water	1000 Ml.

c) Corn - meal - agar medium:-

Corn meal	20 g
Peptone (if desired)	20 g
Dextose (if desired)	20 g
Agar	15 g
Distilled water	1000 Ml.

d) Oat - meal - agar medium:-

Oats	100 g
Agar	15 g
Water	1000 ml.

e) Sabouraud's medium:-

Glucose (or maltose)	40 g
Peptone	10 g
Agar	15 g
Distilled water	1000 ml.

f) Czapek's medium:-

Agar	20 g
Sucrose	30 g
Sodium nitrate	2 g
Potassium dihydrogen phosphate	1 g
Magnesium sulphate $7H_2O$	.5 g
Potassium chloride	.5 g
Ferrous sulphate	.01 g
Distilled water	1000 ml.

Each medium will be prepared by mixing the requisite amounts of the ingredients. It will be sterilized in autoclave at 15 lbs. pressure for 20 -35 mts. The sterilized medium will be poured in sterilized petri dishes and will be inoculated with equal quantity of inoculum.

The growth of the pathogen on the semi solid medium will be measured in centimeters. While, the growth of the pathogen in liquid medium will be measured on the basis of dry weight of mycelium after 21 days of growth. The contents will be filtered through Whatman filter paper No.1 previously dried and weighed. Filter papers containing mycelium will be dried at 60°C for 24 hours in crucibles, after they cool the weight will be taken. All the experiments will be repeated thrice and 5 replications will be used in each case.

Effect of different carbon and nitrogen sources:-

Carbon and Nitrogen requirements will be studied

by replacing glucose and asparagine of the basal medium with different quantities of C & N compounds. The quantity of carbon sources added will be determined on the basis of their molecular formulae, so as to contain an equivalent amount of carbon as present in 10 gm. of the glucose in basal solution. Similarly quantity of nitrogen compounds will be added to the basal solution on the basis of nitrogen present in 1 gm. of asparagine.

Carbon sources to be used:- Xylose, mannose, galactose, Arabinose, Fructose, Glucose, sucrose, maltose, starch, mannitol, sorbitol.

Nitrogen sources to be used:- Ammonium sulphate, Ammonium nitrate, ammonium chloride, Potassium nitrate, sodium nitrate, urea, asperagine, alanine.

Effect of mineral elements:-

Effect of mineral elements on the growth of mycelium will be studied by following the method of Steinberg (1946) with some modifications. $MgSO_4$ will be substituted by $(NH_4)_2SO_4$, $CaCl_2$ by NH_4Cl and $NaNO_3$ by NH_4No_3 respectively.

Effect of temperature:-

150 ml. Erlenmeyer flasks, filled with 50 ml. of sterilized liquid basal medium will be inoculated with an equal amount of fresh inoculum. The flasks will be incubated at 9 different temperatures maintained at intervals of 5°C i.e. 0°C, 5°, 10°, 15°, 20°, 25°, 30°, 35° & 40°C.

Effect of H-ion concentrations on the growth of the Pathogens:-

Erlenmeyer flasks of 150 ml. capacity containing sterilized liquid medium having different P^H will be inoculated with equal amount of mycelium taken out from the fungal colonies. These flasks will be incubated for 15 days at room temperature. The P^H value of the medium will be adjusted by Beckman's P^H meter using N-NaoH and HCl before autoclaving..12 levels of P^H i.e. 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 & 9.0 will be tried.

Biochemical Studies:-

Under this head the post-infection changes caused by the pathogen will be sutdies. Healthy fruits and vegetables inoculated and together with uninoculated fruits and vegetables shall be analysed for sugars, aminoacids, ascorbic acid, phenolics, organic acids and enzymes.

For these studies 5g pulp in the case of AA's 50g pulp from the healthy as well as inoculated fruits and vegetables will be macerated in ground - glass homogenisers containing acid-washed sand and 20 ml. of 80% ethanol. The macerated pulp will be boiled in 20 ml. of 80% ethanol on a water bath. Care will be taken to obtain even the last traces of organic compounds. The solution thus obtained will be kept at a lower temperature, the supernatent thus obtained will be centrifuged at 2,000 rpm for 30 mts. These Studies will be initiated after 3 days of inoculation and will be continued upto 12 day of inoculation.

Sugars:-

One directional chromatography technique (Block et al 1958) will be followed for the estimation of sugars. The solvent system used will be n-butanol: pyridine: water in the ratio of 6:4:3. A straight line curve with a pencil will be drawn on the Whatman filter paper No.1, on this line a diluted extract will be spotted in triplicate by means of a self filling Lame de pipette. In order to keep the spots to a minimum size, they will be dried with the help of an air blower. The chromatograms shall be run for 18 hrs. in the saturated chromatographic chamber with the solvent. The chromatogram will be taken out of the chamber and dried at room temperature till the solvent evaporates. The developing reagent (5 vol. of 4% aniline + 5 vol. of diphenylamine + 1 vol. of orthophosphoric acid) will then be sprayed after this the chromatograms shall be dried at room temperature and then heated at 110°C for 15 mts.

Quantitative estimations:-

Spots from unsprayed chromatograms will be eluted with hot distilled water. By the help of phenol - sulphuric acid method the sugars shall be estimated by using Bausch & Lamb Spectronic - 20 spectrophotometer at wave length of 440 n.m. The quantities shall be determined by referring to a standard curve prepared with authentic samples of sugars.

Free amino acids & organic acids:-

For the estimation of amino acids 2 dimensional ascending technique shall be used. Samples each measuring 30 ml. will be spotted on Whatman paper No.1. The solvents used in this case will be n butanol: acetone: H_2O in the ratio of 120: 30 : 5 followed by liquid phenol: water:ammonia in the ratio of 180:20:1. In each direction the chromatograms shall be run for 18 hrs. and then dried at room temperature after each run. Ninhydrin solution (5%) shall be used as a developer. The sprayed chromatograms shall be left at room temperature for an hour and later heated at 45°C for 10 mts. The coloured spots will be removed and will be read in Bausch & Lomb Spectronic - 20 spectrophotometer at 540 n.m. While proline and asparagine will be read at 440 n.m. and 530 n.m. respectively. The quantities shall be determined by reference to a standard curve prepared with authentic samples of amino acids.

For the estimation of organic acid Uni-directional ascending paper chromatography shall be used. The solvents used in this case shall be n-butanol:formic acid: H_2O in the ratio of 4:1:5. The chromatograms shall be run for 12 hrs. and sprayed with aniline - xylose reagent then heated at 130°C for 20 mts. The spots will be eluted with 70% ethanol. Optical density will be measured at 365 n.m. Quantitative analysis shall be carried out with the help of authentic samples of organic acids.

Enzymes:-

Pulp (10g) from healthy as well as diseased specimens will be macerated in ground glass homogenizer with 20 ml. of 0.2% Nacl solution in distilled water. The homogenate will be filtered through several layers of muslin cloth and squeezed. The extract will then be centrifuged at 400 r.p.m. for 20 mts. and the final volume will be made 25 ml. with 0.2 N Nacl solution. This test solution will the be used for all enzymatic assay.

1. Pectolytic enzymes:-

(A) Pectin - methyl esterase:-

This was carried out by continuous titration method as described by Kertesz (1951), followed by Goodenough & Maw (1974).

For this a solution of 1.2% citrus pectin with P^H adjusted at 5.5 with the help of 1N NaoH will be used as a substrate. 30 ml. of this substrate will be taken in a beaker and to this 4.5 ml. of enzyme preparations will be added. P^H of the reaction mixture will be noted at experimental time zero. After 120 mins. P^H will again be recorded. The original P^H will be recovered by titrating the mixture with .025 N NaoH. The amount of NaoH consumed to get original P^H will be recorded.

The results obtained will be expressed in terms of micro equivalents of methoxy groups released/ml./120 min by the help of the formula suggested by Smith (1958).

Microequivalents of methoxy groups released/ml. = .025N NaOH consumed to get original P^H X .775 / 4.5

(B) Poly-galacturonase (PG)1-

With the help of Viscometric method followed by Hall and Wood (1974) & Bisen & Agarwal (1980) PG will be assayed. Reaction mixture consisting of 1.2% solution of sodium polypectate, 2 ml. of .2 M. will be prepared McIlvain buffer (P^H 4.6), 1 ml. of distilled H_2O and 1 ml. of enzyme preparations. Efflux time will be noted after a definite time interval. The enzyme activity will be expressed in terms of % loss in viscosity and relative enzy activity, the formula of Cappeline (1966) will be applied for the same.

1. % loss in viswcity = $\frac{E_{To} - E_{Tt}}{E_{To} - E_{Tw}} \times 100$

Where E_{To} = Efflux time in seconds for control
E_{Tt} = " " " " " reaction mixture at time t_t"
E_{Tw} = " " " seconds for water.

2. Relative enzy activity (REA)

$= \frac{100}{t \text{ at } V_{50}}$

Where t at V_{50} = Time in mins. required to reduce the viscocity by 50%.

Reaction mixture containing distilled H_2O or boiled enzyme preparations in-stead of active enzy preparations will serve as control.

(C) Polymethyl - galachironase (PMG):-

PMG will also be assayed by the same Viscometric method which was used by Bell et al (1955), Keen & Horton (1966). The reaction mixture will consist of the following composition.

Citrus pectin (1.2%)	2 ml.
.2 M Mollvain buffer ($5\ p^H$)	2 ml.
Distilled H_2O	1 ml.
Enzyme preparation	1 ml.

2. Cellulolylic enzymes:-

Cellulase (CX):- CX enzyme will also be assayed by viscometrical method (Hancock et al 1964 a, Hall & Wood 1974). The composition of the reaction mixture will be as follows:

.5% Cenboxymethyl cellulose	2 ml.
.2 M Mollvain buffer ($p^H 5.5$)	2 ml.
Distilled H_2O	1 ml.
Enzy preparation	1 ml.

The results will be expressed in % loss in viscosity and relative enzyme activity.

Effect of fungicides, phenols & antibiotics on the enzyme activity:-

Effect of various chemicals on the activity of PG, PMG & CX will be investigated by using the same viscometric method. The reaction mixtures will be the same as used previously only in place of distilled water 1 ml. of effective substance of desired concentration will be added. Different concentrations of the following substances will be added:-

(a) Antibiotics:-

		Concentration (ppm)		
(a)	Tetracycline	100	500	1000
(b)	Nystatin	"	"	"
(c)	Chloramphenicol	"	"	"

(b) Fungicides:-

	Concentration (ppm)		
Copper oxychloride	100	500	1000
Brassicol	"	"	"
Morestan	"	"	"

(c) Phenols:-

B- naphthol	"	"	"
Phloro lucinol	"	"	"
Dinitrophenol	"	"	"

Effect of different packing systems:-

To determine the effect of different packing systems on the development of post harvest diseases, the freshly obtained fruits and vegetables will be packed in wooden crate with different kinds of packing material such as wood-straw, rice straw, hay, plastic cuttings, paper cuttings. The fruits before packing will be rinsed with different detergents and sterilising agents. Different combinations of packing material and sterilizing/detergent agents will be tried. After every 3 days the packing will be opened and the intensity of various diseases development will be determined.

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